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PROPERTIES OF THE 'SLOW' SKELETAL MUSCLE FIBRES OF THE FROG*

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It has long been known that skeletal muscles differ widely in their speed of response, their ability to maintain tension, and in many other properties. A number of papers has been published since the middle of the last century dealing with the relation of such characteristics to differences in muscular function. In spite of prolonged and extensive work in this field, however, there was little experimental evidence which showed any wide differences between the physiological properties of skeletal muscles. It remained for Sommerkamp (1928) and later Wachholder and his colleagues (Wachholder & von Ledebur, 1930, 1931; Wachholder & Nothmann, 1932) to show that certain muscles of frogs, and even certain parts of some muscles, exhibited 'tonic' properties not possessed by the other 'non-tonic' muscles of the body. The tonic muscles responded to various stimuli (e.g. to mechanical and electrical excitation, and to immersion in drug solutions) with a slow maintained contraction. The authors did not suppose that the tonic responses were given by qualitatively different fibres; they assumed merely that certain fibres played a dual role and possessed, in addition to the twitch properties held in common with other skeletal muscles, the special quality of responding slowly and with little fatigue.

The stimulus to a more intimate physiological study of striated muscles was given by the findings reported in previous papers that slow contractile responses were evoked in some frog muscles by stimulation of motor nerves of small diameter. Now that it has been established (Kuffler & Vaughan Williams, 1953) that there are two functionally distinct nerve-muscle systems (the small-nerve slow-muscle fibre system, and the large-nerve twitch-fibre system) a detailed examination of the different properties of muscles which do and do not contain slow muscle fibres has become physiologically more meaningful.

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The twitch-producing muscle fibres, as a group, have in common a set of properties, among which are included definitively the ability to give on nerve stimulation propagated electrical impulses associated with a reversal of membrane potential ('overshoot'), and the well-known twitches, i.e. fast shortening or tension development. There exist many differences within this group, such as variations in colour, transparency, rates of conduction, contractile behaviour and even in histological characteristics. These differences, however, might be termed quantitative. The 'slow' muscle fibres, on the other hand, differ from the twitch fibres so fundamentally that they may be regarded as qualitatively distinct elements. The twitch response and propagated impulse are absent. In addition, the slow fibre membrane properties are in a separate class, since on nervous excitation the action potential does not exceed the resting potential, nor is the restitution process after an initial depolarization similar to the local responses which can be set up in twitch fibres by subthreshold stimulation. Further, the innervation of slow muscle fibres is diffusely distributed over the whole fibre length, in contrast to the discrete focal pattern of twitch fibres. There is also evidence that twitch and slow muscle fibres can be distinguished histologically (Krüger, 1952). It is likely that many other differences from twitch fibres will emerge, such as peculiarities in ionic content and exchange, or in metabolic requirements.

The existence of qualitatively distinct slow elements in skeletal muscle raises the question as to how far previously observed phenomena can be attributed solely to their activity. In the present study the distribution of the slow fibres in the body and their approximate density in some of the muscles has been investigated. Evidence will be presented that it is they alone which respond to immersion in solutions of acetylcholine (ACh) or KCl with a prolonged contraction, so that all pharmacological assays of ACh on, for example, the frog rectus preparation have in fact been carried out upon the slow fibre system. It is thought that many results obtained in previous studies dealing with 'tone' or 'contractures' in frog skeletal muscles can be interpreted as the responses of the two distinct types of muscle elements. For the reviews of this extensive field see Fulton (1926), Gasser (1930), Bremer (1932), Brecht (1952) and Krüger (1952).

METHODS

As in previous experiments male and female summer and winter frogs (*Rana pipiens*) and bullfrogs (*R. catesbiana*) were used. Although seasonal differences may play a role, they did not affect the fundamental properties of the muscles as far as this study is concerned.

Large or small motor nerve fibres were stimulated selectively by the method described in the preceding paper (Kuffler & Vaughan Williams, 1953). Tensions were measured isometrically (unless otherwise stated) by a mechanico-electrical transducer, RCA 5734 tube, coupled to a D.C. amplifier. Solutions bathing the preparations were conveniently changed with minimal interference to the muscle by apparatus illustrated in Fig. 1. The muscle lay in a small open Perspex container holding frog Ringer solution. The container in turn lay in a larger dish holding paraffin oil. By a

system of communicating vessels the solution surrounding the muscles was removed and simultaneously replaced by the test solution. For electrical recording with external leads the muscle was lifted into the paraffin oil, while for tension measurement or intracellular recording it could be left in position in the bath.

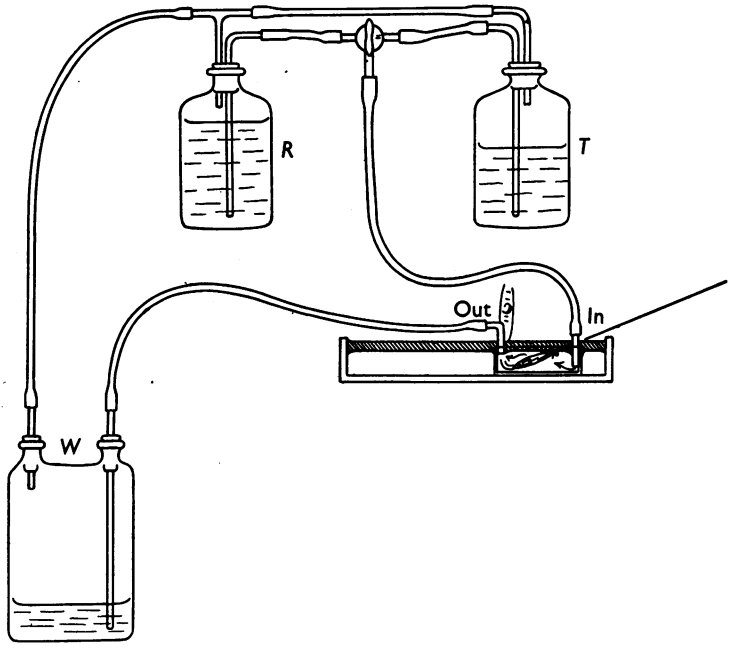

Fig. 1. System of communicating vessels for removing and simultaneously replacing solutions surrounding muscles. *R*, Ringer; *T*, test solution; *W*, waste. Shaded area: paraffin oil.

RESULTS

Characteristics of tension development on small-nerve or 'direct' stimulation

The characteristic contractile response to stimulation of a single small-nerve fibre was reported by Tasaki & Mizutani (1944), and detailed observations were published by Kuffler, Laporte & Ransmeier (1947) and Kuffler (1949). With the present convenient method of blocking large-nerve fibres many further tests which required repeated controlled stimulation were performed. The method made possible the separate and repeated activation of two different groups of muscle fibres, so that a study could be made of the individual behaviour of each group and of their interaction.

Time course of tension development. Facilitation. If one or a few small-nerve (s.n.) fibres are stimulated with a single shock, no muscle movement or only a minute movement is seen in muscles of the size of the iliofibularis or semitendinosus. Nevertheless, with low initial muscle tensions (about 0·5 g or less) and stimulation of practically all s.n. fibres to such muscles, the mechanical

effect of one stimulus can always be detected visually or by a sensitive tension measuring instrument. Slow muscle fibres show true mechanical facilitation, i.e. during a series of stimuli successive nerve volleys add increasing amounts of tension. Fig. 2 illustrates the increasing effectiveness of s.n. stimulation as the frequency increased. It is of interest to note that almost the same tension is produced by stimulation at 40/sec as by stimulation at 75/sec or at higher rates. The principal difference lies in the speed at which the tension is developed. These curves resemble those in crustaceans (Katz, 1936; Pantin, 1936) where non-propagated responses predominate.

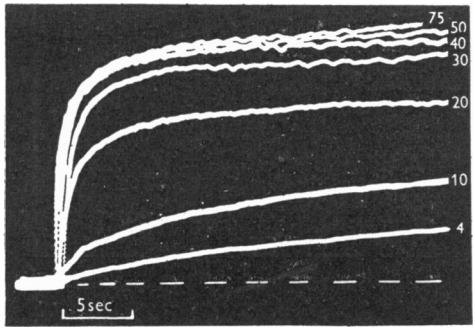

Fig. 2.

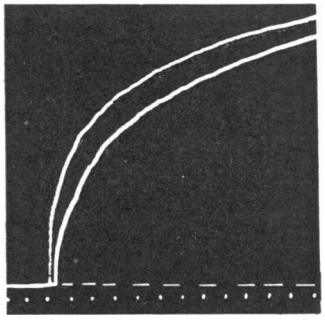

Fig. 3.

Fig. 2. Effectiveness of small-nerve (s.n.) stimulation at various frequencies. Isometric tensions set up in slow muscle fibres in the iliofibularis by stimulation at frequencies of 4, 10, 20, 30, 40, 50 and 75 per sec, maintained for the duration of the sweep. No twitch motor unit activity present. All seven exposures superimposed on one record. At the three highest frequencies the final tension was similar but the rate of rise differed. Maximum tension 2 g. Initial tension (I.T.) 0·5 g. Transducer set-up.

Fig. 3. Isometric tension developed by slow muscle fibres of the iliofibularis on s.n. stimulation. Lower record: stimulation at 20/sec for duration of sweep. Upper record stimulation at 10/sec with paired stimuli 16 msec apart. Note the faster tension rise. I.T. 0·5 g. Maximum tension 1·6 g. Time scale, 1·8 sec intervals.

Fig. 3 illustrates a mechanism of speeding up the rate of slow muscle fibre tension development by closely spaced double stimuli. Paired volleys 16 msec apart were more effective at a rate of 10/sec (steeper curve) than single stimuli at 20/sec, although no consistent difference between the final peak tensions was seen. In view of the dense innervation of slow muscle fibres and the known synchronous bursts of s.n. discharges during reflex activity, it is likely that the frog can make use of such a mechanism in its normal behaviour. Shorter bursts of 'paired' discharges would be as effective as longer discharges of relatively high frequency, regularly spaced.

It is difficult to make strict comparisons between the rates of tension development between twitch and slow fibres, since conditions which are optimal

for the one are not for the other. Large tensions rise to a peak in 20–40 msec at about 22° C in the sartorius and other muscles after a single nerve stimulus, while the slow fibres must be repeatedly stimulated for effective tension development. The rate of tension rise in slow fibres recorded at the muscle tendon under isometric conditions can nevertheless be quite rapid on tetanic stimulation, although it is always many times slower than that of the twitch elements in the same muscle.

The rates of relaxation of the two groups of muscle elements are even more strikingly different. At temperatures around 20° C the tension after a twitch

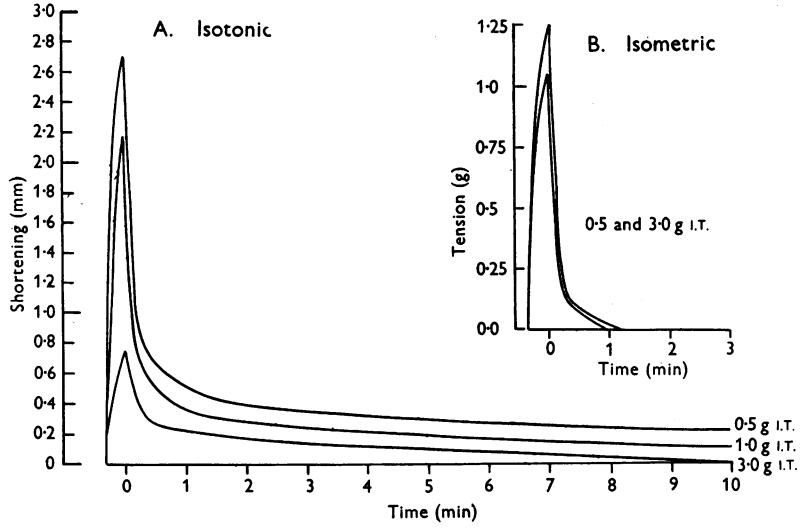

Fig. 4. Slow fibre activity recorded isometrically and isotonically from a 'slow' strip preparation of iliofibularis. A, isotonic. Small-nerve stimulation for 20 sec at a rate of 20/sec. Note the well-maintained residual shortening 10 min after cessation of stimulation at I.T. 0·5 and 1·0 g. B, isometric. Stimulation as in A, same preparation. Note there was little difference in tension peak and time course at the two initial tensions.

tetanus usually falls to the base-line in a fraction of a second, while after slow muscle fibre activity the relaxation is measured in many seconds or minutes (Fig. 4*A*, *B*).

Effect of initial tension and of recording system. The optimal initial tension for slow fibre tension development in muscles of the size of the iliofibularis is between 0·3 and 0·7 g. For twitch fibres of this muscle, on the other hand, the optimal initial tension is between 1·5 and 3 g. In this connexion it is of interest that at an initial tension of less than 1 g there is some failure of large-nerve twitch fibre transmission in many frog muscles, even in the absence of fatigue or injury (Kuffler, 1952).

The RCA transducer, giving full-scale deflexion to a stylus movement of only 80 μ, did not contribute effectively to the muscle shortening. Whatever

shortening occurred, therefore, was that permitted by the elasticity of the muscle itself, and of the tendon and thread. If shortening is permitted, slow fibres relax even more slowly than under isometric conditions. The rates of relaxation after cessation of maximal s.n. stimulation at similar initial tensions are compared under isometric and isotonic conditions in Fig. 4. The small nerves innervating the muscle were stimulated for 20 sec at a rate of 20/sec. The long-lasting residual shortening at low initial tensions (0·5 and 1·0 g) is striking. This residual tension, persisting after stimulation has ceased, can be immediately abolished or greatly reduced by stretch or by a superimposed twitch (see below, Fig. 6*b*). The importance of initial tension and recording conditions for the demonstration of slow fibre effects is emphasized, for these factors are significant in the interpretation of work on contractures, particularly by earlier workers who preferentially measured shortening of muscles (see discussion).

There is some evidence that even in the absence of stimulation the slow fibres 'spontaneously' contract to a certain length. If the slow fibre region, in muscles like the iliofibularis or semitendinosus submerged in Ringer solution, is closely observed microscopically under transmitted illumination, a gradual shortening and 'bunching' of that area is frequently seen without any nerve excitation. If the muscle is stretched and released again, the shortening reappears slowly. The stretch itself may be a stimulus. This 'spontaneous' activity is mentioned since it has frequently been observed, but no definite evidence supporting these impressions and excluding various other causes (e.g. small injuries) is available.

The interaction of slow and twitch fibres. Previous attempts to demonstrate the summing of twitch tensions and slow muscle fibre tension were not always successful. These failures were mainly due to inadequate mechanical recording conditions. When small nerves were stimulated by the present methods, it could be readily demonstrated that the tension produced would sum with that produced in response to a superimposed stimulation of twitch fibres. To demonstrate this point the large-nerve fibres of root 10 were stimulated in Fig. 5 and after the first two twitches s.n. stimulation was started. Addition of root 9, which generally contains the majority of large-nerve fibres, but only few s.n. elements (Fig. 10*b*), merely increased the twitch height which was added to the slow tension.

The other main points of interest in the interaction of the two systems are as follows. If a brief period of twitch fibre stimulation (preferably submaximal) is superimposed upon a maximal s.n. tetanus, the slow fibres may maintain a greater final tension than they could have developed alone (Fig. 6*a*). A corollary of this phenomenon is that after a brief superimposed twitch tetanus, continued slow fibre stimulation at a low frequency (producing little tension by itself) will maintain a tension that would otherwise only have been reached by slow fibre stimulation at higher frequencies.

In contrast to the synergism of twitch and slow muscle fibres *during* s.n. activation, the slow relaxation *after* the completion of a period of s.n. stimulation can be greatly hastened by the interposition of brief twitch fibre stimulation. Fig. 6*b* illustrates how this slow fibre 'residual tension' is suddenly reduced (collapsed) by a short tetanus. It was this phenomenon which formerly encouraged the belief that fast and slow tensions were produced by the same muscle elements. The effect is most probably purely mechanical, because slow fibre residual tension can also be abolished by stretch.

'*Direct*' *stimulation of slow muscle fibres.* The characteristic response of muscles containing slow fibres is exhibited not only when small motor nerves are stimulated, but also when a muscle containing them is stimulated directly. 'Direct' excitability of muscle fibres was usually tested in the following way.

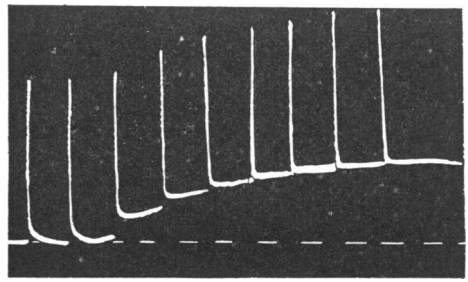

Fig. 5. Summation of tension of slow and of twitch muscle fibres. Large-nerve fibres arising in root 10 to the iliofibularis were stimulated at a rate of 1/sec causing twitches of 2·5 g. After the first two twitches s.n. stimulation was started in the root by a second pair of electrodes. The twitch tension sums with the tension in the slow muscle fibres. If the nerve fibres in root 9 were also excited the twitches which were added to the slow tension became much larger.

A curarized iliofibularis muscle was divided longitudinally by careful dissection into two equal parts, an operation which can be performed with damage to only a few fibres. One part included the slow muscle fibres innervated by small nerves, the other contained twitch elements only and was supplied by the larger nerve fibre group. Even in the slow portion, however, the slow fibres were always outnumbered by twitch fibres, the slow fibres being merely interspersed among them (Kuffler & Vaughan Williams, 1953), and contrary to widespread belief there have been found no muscles or parts of muscles containing 'purely tonic' elements only.

Direct stimulation through two Ag-AgCl-wick electrodes applied to the surface, using rectangular current pulses of 1·0–10 msec duration, resulted in two different types of response depending upon which of the two parts was tested. The strip without s.n. innervation gave simple twitches and propagated action potentials only and when single stimuli were made very strong (several

times maximal) some of the muscle fibres occasionally responded with repeated discharges. This could be detected electrically and at the same time the tension record became broader. If stimuli were further increased, a residual shortening followed on some occasions. This, however, was clearly an effect of injury, since also microscopically visible changes resulted (opaqueness around the cathode), and subsequent excitation became less effective.

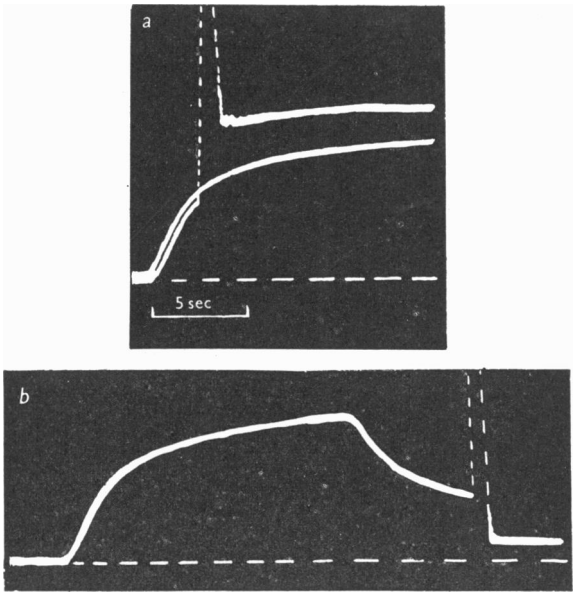

Fig. 6. Interaction between slow and twitch fibres in the iliofibularis. *a*, two tension records superimposed. Small-nerve stimulation at 30/sec causes a slow smooth tension rise reaching peak value of 0·6 g. In the second exposure a short burst of twitch fibre activity is added (tension off the screen) during continued s.n. stimulation. After the tetanus the slow muscle fibre activity maintains a higher final tension value (0·75 g) than it would have reached without the interposed twitch tetanus. *b*, s.n. stimulation at 30/sec sets up tension of 0·8 g in 20 sec. During slow relaxation following cessation of s.n. excitation a short tetanus in twitch fibres causes a collapse of most of the residual tension. I.T. 0·5 g in *a* and *b*.

In the strip containing slow fibres, the initial effect was also a twitch, but with increasing strength, at a definite threshold, a residual shortening followed, without preceding repetitive discharges (Fig. 7). The height of the twitch peak was not necessarily affected, when the threshold for residual tension was reached. The residual tension lasted for many seconds, or minutes, depending upon initial muscle tension and upon mechanical recording conditions. When more shortening was allowed, the observed effect was bigger. This residual tension behaved in most respects like the tension after cessation of s.n. excitation. It was diminished or abolished by transient stretch or by muscle

twitches and could be repeated without obvious injury. It occurred at current strengths which did not cause injury in twitch muscle portions of comparable size. The residual tension summed when series of strong direct stimuli were given and the twitch tensions were generally added to the slow tensions in a similar manner as is seen in Fig. 5 with stimulation of the large and s.n. innervation. This suggests that different muscle fibres were involved in the twitch and slow responses. During prolonged trains of strong stimuli, however, the twitch height frequently declined, probably an effect of anodal blockage and/or twitch fibre contractures at the cathode. Unlike slow muscle fibre excitation through the s.n. route, frequent repetition of direct stimulation resulted in 'fatigue' of the residual tension effect. Sartorius muscles or adductor muscles, strips or whole preparations, behaved similarly to the quick twitch

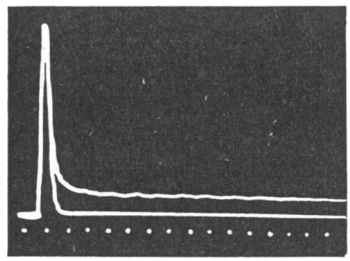

Fig. 7. Direct excitation of slow muscle fibres in the 'slow' portion of the iliofibularis. Two different strengths of stimulation, tension records superimposed. Peak twitch tensions are similar, but stronger stimulus is followed by a prolonged tension residue. Such residual tension was absent if the twitch portion (containing no, or few, slow fibres) of this muscle was stimulated. Peak tension 7 g; I.T. 1·0 g, time, 10 c/s.

portion of the iliofibularis; i.e. the characteristic slow-fibre residual tension was absent.

It appears that in the 'slow' strip of the iliofibularis there exist some muscle fibres which show different contractile properties from those in the pure twitch portions. Since these contractile qualities resemble those found in slow fibres, reliably identified by intracellular recording techniques, one may conclude that the same elements are responding with slow contraction to direct excitation and s.n. stimulation. Attempts to obtain more detailed information by isolating single slow fibres were unsuccessful, nor was it possible to pass current through a single fibre with one internal electrode while recording the response with another in the same fibre. This can be done only with the twitch muscle fibres which even in the slow portion are more numerous and stand out on the surface.

The 'direct' stimulation experiments, as well as the other tests described below, confirm essentially the clear-cut earlier results of Sommerkamp (1928),

who confined the slow responses of tonic fibres to an anatomically distinct 'tonus bundle'. He supposed, however, that both fast and slow responses were given by the same elements. Our own findings show that the fast responses of the bundle are given by twitch fibres, among which the slow fibres are interspersed. This mingling of fibres is in agreement with the histological data of Krüger (1952) and Günther (1949).

Specific 'chemical' and electrical properties of slow and twitch muscle fibres

'Chemical' properties. According to their behaviour in response to acetylcholine (ACh) or occasionally some other agents, muscles were divided into 'tonic' or 'non-tonic' groups (Riesser & Richter, 1925; Sommerkamp, 1928; Wachholder & von Ledebur, 1930; and others). Slow maintained shortening with injection or application of drugs was the criterion of the 'tonic' property. The 'non-tonic' muscles were those which gave only a transient shortening.

These older findings were re-examined in the light of present evidence of large and s.n. innervation patterns and the distribution of slow muscle elements within muscles. It was found that only those muscles which contained s.n. innervation gave maintained tensions when immersed in solutions of ACh-Ringer or KCl. Other muscles, and the large-nerve innervated portions of the iliofibularis and semitendinosus, produced only transient tensions in similar solutions.

A typical experiment is shown in Fig. 8*A*. Immersion of the slow iliofibularis strip into ACh 10^{-5} in Ringer solution (Fig. 8*A*, *a*) resulted in a relatively rapid initial tension development (isometric recording) accompanied by visible twitches of the muscle. Within the first minute the tension fell to 1·7 g and thereafter gradually declined during the following half an hour. On the other hand, muscles which contained predominantly twitch fibres, for instance the sartorius, adductor longus or the twitch portion of the same muscle (iliofibularis), exhibit the first phase of tension development only. A representative experiment, illustrated by record *b* of Fig. 8*A*, shows the response of the adductor longus to immersion in isotonic KCl. In spite of the continued presence of the depolarizing solution, the muscle was fully relaxed within 3 min.

Just as the slow response to direct stimulation was correlated with the presence of small-nerve innervation, the slow development and prolonged maintenance of tension in solutions of depolarizing drugs was found in all muscles which received s.n. innervation, and in no others. Here again the assumption seems reasonable that it is the distinct slow fibre elements alone which produce the long-maintained tension. A list of muscles containing slow fibres is given in Table 1. Muscles which exhibited a behaviour similar to the adductor longus or to the twitch portion of the iliofibularis are presumed to contain no, or very few, slow fibres.

The most likely places of attack of substances like ACh on slow muscle fibres are the junctional regions. While KCl depolarizes twitch fibres along their whole course, ACh and some other substances depolarize the end-plate regions only (Kuffler, 1943). In line with this observation, curarine which also acts

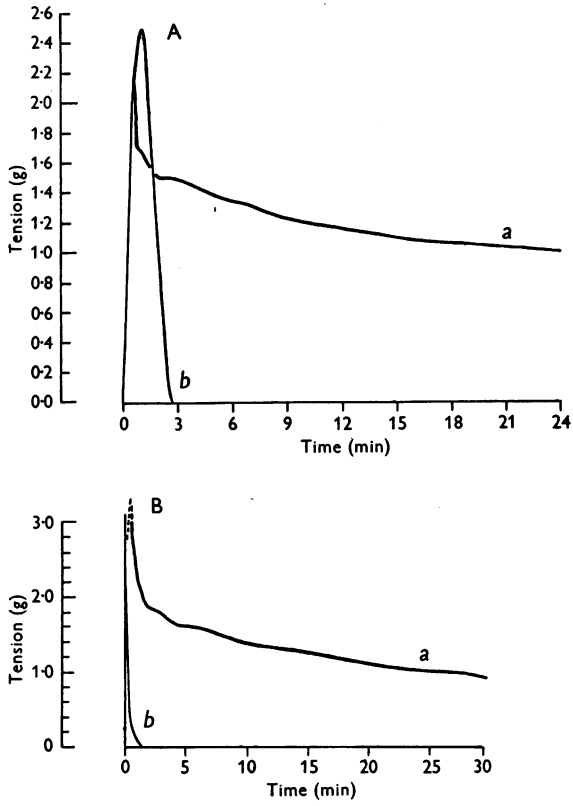

Fig. 8. Isometrically recorded tensions during chemical and electrical excitation of slow and twitch fibres. A, chemical excitation. *a*, slow portion of the iliofibularis immersed in 10^{-5} ACh. Within the first minute there occurred noticeable transient propagated twitch fibre activity, followed by a gradual tension decline. I.T. 0·5 g. *b*, adductor longus in isotonic KCl develops transient tension rise only. I.T. 1·0 g. B, electrical excitation. *a*, slow iliofibularis portion during constant current flow of 50 μA maintained some tension for 30 min. I.T. 0·5 g. *b*, current of 180 μA passed through adductor longus sets up transient tension rise only. I.T. 1·0 g. Twitch portion of iliofibularis gave similar tension curves as the adductor longus. Large tensions which occur during the first 30 sec of drug action or of current flow are not plotted.

specifically at the end-plate, antagonizes the depolarizing action of ACh but not that of KCl. Correspondingly the contractile response to ACh is abolished by curarine, but the response to KCl is not affected. The same holds for slow muscle fibres, since the addition of curarine will diminish or prevent ACh contractures but not KCl contractures. One may assume that since the slow

muscle fibres possess numerous and diffusely distributed junctional regions, the action of ACh also is exerted along the whole length of the muscle fibre.

Reaction to constant current. The effect of constant current on muscles has been studied for over 100 years, and it is well known that at the cathode first a tetanus, i.e. a burst of propagated responses, is set up, followed by local shortening, or contracture. The time course of the cathodal shortening, however, was rarely followed for long periods. In the present experiments the behaviour of certain groups of muscles to constant current was found strikingly similar. In short, if the current (usually 40–80 μA, depending on shunts) applied through non-polarizable electrodes was maintained, the shortening in the s.n. innervated muscles was also maintained for long periods (Fig. 8*B*, *a*). The twitch muscles, however, under comparable conditions relaxed completely, even with much higher currents, usually within a minute (Fig. 8*B*, *b*).

The important difference between the twitch and slow elements is in the length of time a contracture can be maintained in the presence of a continuing stimulus, whether it be constant current or a depolarizing drug. Thus, contractures lasting some seconds can be demonstrated in twitch fibres also, and can be made to mirror fluctuations of current with corresponding variations in tension. No predominantly twitch-fibre muscle, however, can be made to maintain a tension of several minutes duration in response to such stimuli.

The time course of 'active' tension development. It has already been mentioned that the residual tension after cessation of s.n. stimulation was collapsed by twitches (Fig. 6*b*) or by a brief stretch. When muscles containing slow muscle fibres were immersed into drugs, a similar collapse followed immediately after twitches or on release from stretch. The collapse, however, was temporary and at least part of the tension gradually redeveloped at a similar rate as the original tension rise. This was taken as an indication that some active process was still present which continued to provoke tension after a transient abolition. The phenomenon was observed during contractures caused not only by drugs but also by constant currents.

Fig. 9*A* illustrates the slow muscle fibre tension time course recorded isometrically from a whole iliofibularis during immersion into 10^{-5} ACh in Ringer solution. During the first tension plot (Fig. 9*A*, 1) the muscle was stretched for 3 sec every 3 min by a weight of 4 g. It took about 3 min after each stretch for the tension to rise and to level off again at a somewhat lower value. No stretch was applied between 36 and 48 min. After a 30 min period of rinsing in Ringer solution, the ACh effect was again recorded for 46 min, after which a single stretch was applied (Fig. 9*A*, 2). It is clear that the rates of fall of tension and even the absolute values were similar in both tests.

In Fig. 9*B* the muscle shortening was recorded from a small strip preparation containing the slow muscle fibre portion of the iliofibularis, during immersion into 0·45% KCl in Ringer solution. The difference between the curves, with

and without stretch, is striking and confirms that shortening under isotonic conditions does not correlate well with the active maintenance of tension. In curve 2, for instance, the muscle was still shortened by 11 mm after 38 min.

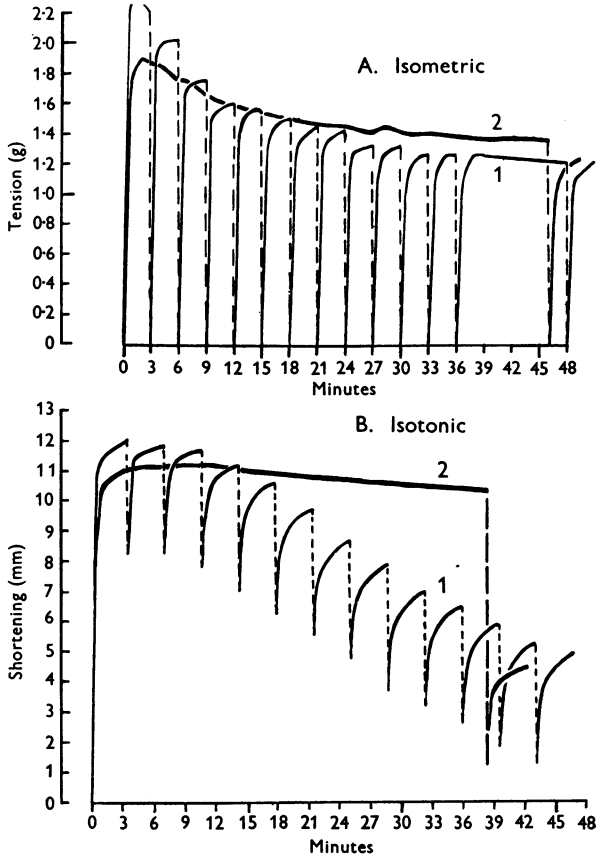

Fig. 9. Time course of 'active' process in slow fibres. A, immersion of whole iliofibularis in 10^{-5} ACh, isometric recording. In curve 1 the muscle was stretched by a 4 g weight every 3 min for 3 sec, causing a transient tension collapse. During the intervals most of the tension was restored. In curve 2 the same ACh effect was recorded and stretch was applied only once after 46 min. The time course of tension decline was similar in both curves. I.T. 1·5 g. B, isotonic recording from slow portion of iliofibularis. Immersion into 0·45% KCl in Ringer solution. The shortening was well maintained for 39 min in curve 2 until a stretch of 2 g was applied for 3 sec. Only the 'active' component of the shortening was restored. Curve 1, with 2 g stretches interposed at 3 min intervals, gives the estimated time course of the 'active' maintenance of shortening. Since the muscle was smaller than in A, the I.T. was 0·5 g and the applied stretches were only 2 g.

The shortening was reduced to 5 mm by an applied stretch of 2 g, i.e. the major portion was 'mechanical' effect. This observation appears similar to Hess's (1927) 'Löschphänomen' during ACh contraction. In other preparations, and

particularly when strong concentrations of KCl were used, the contrast was not as great, but on the whole the time course of isometrically recorded tension (irrespective of periodic stretch) was similar to the course of isotonic shortening with interposed stretches.

It is thought on the basis of these experiments that the slow muscle fibre activity has two components. The first is a passive 'holding' action represented by residual tensions which can largely be collapsed. It is seen either after cessation of s.n. stimulation (Fig. 6*b*) or during the plateau of shortening (Fig. 9*B*, 2) recorded isotonically. The 'holding' seems the result of 'friction', determined by some physical characteristics of slow muscle fibres and other mechanical factors which keep muscle elements 'in place'. In support of the latter assumption is the observation that in well-cleaned small strips of muscles from which most of the connective tissue has been removed, the residual tensions following s.n. stimulation are smaller and briefer. The second is an 'active' component which can restore tension or shortening after a temporary collapse. Evidence suggests that this active continuance of tension development is controlled by changes of membrane potential. Only a reconstitution of the 'resting' membrane charge, e.g. by reversing the depolarization by anodic polarization, will reduce the muscle tension. If current was passed through a muscle during drug depolarization, the tension was further increased at the cathode and decreased at the anode. This phenomenon of contracture reversal was previously studied in twitch muscle fibres by Kuffler (1946) and in the rectus muscle by Fleckenstein, Hille & Adam (1951) and correlated with the membrane change.

The difference between the contractile behaviour of slow and twitch muscle fibres can only be regarded as quantitative, in contrast to the wide dissimilarity between their membrane characteristics. Twitch muscles always relax relatively rapidly in spite of continued membrane depolarization. The contractile elements of slow fibres also 'accommodate' to depolarization, but the process is fifty to a hundred times slower.

Distribution of slow muscle fibres in the body

Maintained shortening with ACh and KCl solutions has been found a good indication of the occurrence of s.n. innervation and of the existence of slow muscle fibres. This is, in fact, the same test as used for many years for the detection of 'tonic' muscles. In some of these muscles the grouping of the nerve spectrum was checked electrically and in others an approximate estimate of the slow muscle fibre density was obtained from the values of drug contractures in relation to twitch tensions. In Table 1 the wide distribution of the s.n. slow fibre system is demonstrated. No attempt was made to study all muscles systematically, although the occurrence of slow fibres in functionally related groups would be of interest. In Table 2 information concerning small

TABLE 1. Distribution of slow fibres in the body

Upper limb		Lower limb		Other muscles	
Muscles containing slow fibres	Muscles containing predominantly fast fibres	Muscles containing slow fibres	Muscles containing predominantly fast fibres	Muscles containing slow fibres	Muscles containing predominantly fast fibres
Sternoradialis	Deltoideus	Iliofibularis + + +	Sartorius	Extrinsic muscles of the eye	Depressor maxillae
Infraspinatus	.	Semitendinosus + + +	Adductor longus	Rectus abdominis + + + +	Tensor fasciae latae
Levator scapuli	.	Gastrocnemius	Gracilis	Pectoralis abdominis (both portions)	.
Transversus scapularis major and minor	.	Semimembranosus	Peroneus	.	.
Flexor digitorum communis	.	Tibialis medialis	Tibialis lateralis	.	.
.	.	Extensor longus dig. IV	.	.	.

TABLE 2. Distribution of small motor nerves to lower limb. Ventral roots which supply fast and slow fibres to muscles of the lower limb

		Motor roots supplying muscle					
		8		9		10	
Nerve to muscle	No. of records	Fast	Slow	Fast	Slow	Fast	Slow
Iliofibularis	30	−	−	+ + +	+	+ +	+ +
		.	.	.	+ + (in 2/30)	.	− (in 2/30)
Semitendinosus	5						
Anterior		−	−	+ +	+ +	−	−
Posterior		−	−	+ +	+ +	−	−
Gastrocnemius	2	.	.	+ + +	−	+ + +	+ upper part only
Sartorius	3	+ + +	±	+ + +	+	−	−
Nerve to gluteus and cruralis	1	−	−	+ +	−	+	±
Gracilis	2	−	−	+ + +	−	−	−
Peroneus	2						
Longus		.	.	+	−	+ + +	±
Brevis		.	.	+	−	+	−
Tibialis							
Lateral	1	.	.	−	−	+ +	−
Medial	1	.	.	+ +	+	.	.
Ext. long. dig. IV	1	+ +	+	+	+	.	.

and large motor nerve fibre contributions of different roots, derived from conduction velocities, is given for several muscles. Appreciable variations in different frogs have been observed. Fig. 10 illustrates the types of record from which Table 2 has been constructed. No numerical estimate of the proportion of small to large nerves has been made. Comparison of size in potentials representing the two fibre groups gives only a very crude approximation of the actual number of fibres.

DISCUSSION

In the previous study it was demonstrated that two groups of muscle fibres of quite distinct character exist in frog skeletal muscles: the twitch fibre group, innervated by large-nerve (8–40 m/sec conduction velocity) and the slow fibre group, innervated by small-nerve fibres (2–8 m/sec). The present study, con-

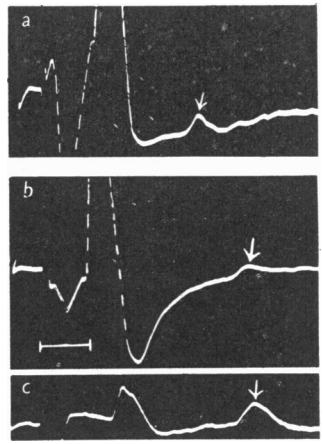

Fig. 10. Small- and large-nerve fibre components in motor nerves to muscles. *a*, maximal single stimulus to ventral root 8, recording from nerve to the sartorius before its entry into the muscle. Only a few relatively slow high-threshold small-nerve impulses (arrow) reach this muscle. Faster large-nerve impulse potentials off screen. *b*, stimulation of ventral root 9, recording from iliofibularis nerve. Small-nerve impulses (arrow) in this root are not numerous. *c*, ventral root 10 to iliofibularis contains most of the small-nerve fibres going to the slow portion of the muscle, relatively few of the faster conducting large-nerve group. Time, 1 msec.

cerned with the performance of slow muscle fibres under a variety of conditions, gives us, first of all, some indication of the mechanics of activation. A local graded contraction, if it were confined to a small section of a muscle fibre, would be very inefficient, since much of the energy would be expended in stretching the series elastic elements of the inactive portion of the muscle. In a twitch fibre a propagated wave of activation spreads rapidly in both directions from the neuromuscular junction, so that the whole fibre contracts within a few milliseconds, largely eliminating the effect of series elasticity within the fibre. In slow fibres propagation is absent, yet the whole fibre contracts almost

simultaneously because its different parts are individually stimulated by the numerous neuromuscular junctions distributed over the whole fibre length.

The activation of slow fibres can be graded very finely by variations in the frequency of s.n. fibre discharges (Fig. 2). The 'slow' fibres can produce a wide range of tensions, from a scarcely perceptible contraction at low frequency to relatively quick and strong action during a rapid tetanus. In addition, the slow muscle fibres stimulated through their nerve supply are much more resistant to fatigue than the twitch fibres. They seem, therefore, well suited for prolonged maintained activity. During the latter the greater economy of local as compared with propagated activity becomes important. During each twitch a muscle fibre comes abruptly and explosively to full activity (Hill, 1949), while under conditions of graded local activation an energetically less expensive, although slower, adjustment to requirements can be made.

No accurate data about the tension developed by individual fibres could be obtained. The largest isometric tension observed upon small-nerve stimulation was 2·7 g produced by an iliofibularis (total wet weight 70 mg). The same muscle gave a tension of 20 g upon a single volley to all the twitch fibres (28° C). Since the relative number of twitch and slow fibres is not known, an accurate estimate of their respective performances cannot be made. From the frequency with which fibres of each type were entered upon random penetration with microelectrodes, it appears that even in the regions of the greatest density of slow fibres they are out-numbered by twitch fibres. Isotonic recording favours the demonstration of slow muscle fibre activity; twitch fibres, on the other hand, develop more tension the more isometric the record. Further, quite different initial tensions are optimal for the two groups, so that in any interpretation of contractions as due to slow or twitch elements, careful attention has to be paid to the recording conditions.

Experiments on fibres of the lizard (Buchthal & Lindhard, 1939) and on single frog muscle fibres (Kuffler, 1943) established that application of ACh to the surface of a twitch fibre had no effect except in the region of the nerve-muscle junction. Even when ACh was applied at this point, the frog fibre responded with a few twitches only and, depending on the concentration, with a short but not with a prolonged contracture. It was thus difficult to understand why many frog muscles nevertheless produced large maintained contractions upon immersion in solutions of ACh. The phenomenon is now readily explained in the knowledge that only the slow fibres exhibit a maintained response, and that numerous junctional regions are scattered over the whole surface of each individual slow fibre.

Differences in ACh effectiveness in 'tonic' and 'phasic' muscles were recently studied by Brecht & Feneis (1950). The authors believe that there need not exist striking differences in the time course of the contractile response in individual fibres in 'tonic' and 'non-tonic' muscles. Individual fibres or

portions of 'tonic' muscles always gave a much faster tension decay during immersion into ACh, than the whole muscle. They have clearly shown that diffusion into the muscle interior takes a long time, and some accommodation to the slowly rising drug concentration occurs in the muscle interior. Brecht & Feneis also suggest that ACh may be inactivated more quickly in 'phasic' muscles. These factors, as well as the amount of connective tissue present in different muscles, are likely to play a role in determining the response to ACh (see also Rössel, 1951). In the present experiments on the iliofibularis, however, muscle parts of comparable size were used and differences in diffusion conditions could hardly be significant in all experiments. Further, the different response types were equally found with constant currents, which eliminates the factor of diffusion, and with KCl, which eliminates the rate of destruction of ACh. Brecht & Feneis in their study correctly conclude that no dual contractile mechanisms in the same muscle fibres need to be postulated to explain the phenomena of ACh action. It has to be recalled, however, that twitch fibres as well as slow fibres can give 'contractures', and that the differences in the contractile behaviour are of a quantitative nature only.

Contracture. Accepting Gasser's (1930) restrictions on the term, contractures may be defined as reversible, prolonged, not-conducted contractions. It is suggested that contractures in skeletal muscles may be divided into two main groups.

The first consists of prolonged contractions caused by stimulation of slow fibres. Evidence has been obtained that it is the slow fibres alone which give a maintained contraction in response to a direct stimulation by long-lasting currents or even short pulses (Figs. 7, 8 *B*, *a*) of non-traumatic strength. In this category can probably be included many contractures which have been classed as Tiegel's contractures. These were originally observed in frog muscles, and only upon direct stimulation with strong currents. The well-known contractures first described by Bremer (1928) in frogs were best obtained with two stimuli separated by a critical interval, and were also found after neuromuscular block. Recently, Bremer & Desmedt (1947) detected in these 'contractions pseudoveratriniques' repetitive discharges in twitch fibres and these findings suggest that the phenomena are not related to slow fibre activity.

The second group consists of reversible contractures of twitch fibres, elicited by direct current or drug solutions like ACh or KCl. The important characteristics of these contractures of twitch fibres is that they are of relatively brief duration, a few minutes at most. The twitch fibres appear to possess a mechanism, less pronounced in the slow fibres, which accommodates them to the presence of a continuing stimulus, so that the contractile process is no longer activated. The contractile elements thus are readily dissociated from the membrane depolarization (produced by outflowing, cathodal, current or other agents) which provides the first step in initiating the contractile process.

Another group, not strictly within the above definition of contractures, may be the long-lasting shortening seen after the passage of very strong electric currents. These contractures are never quite reversible and there generally follows visible evidence of damage. They are, therefore, pathological and should not be regarded as physiological contractures. In this connexion it is of interest to recall the increased sensitivity to the development of contractures in denervated muscle, see e.g. Brown (1937). Contractures, not normally seen, are also observed clinically in a variety of diseases. It may be speculated that the slow muscle fibres represent a more 'primitive' skeletal muscle type which has been superseded by the more efficient twitch fibres carrying conducted impulses. During pathological conditions some of the 'hidden' properties may emerge.

The mechanism by which the contractile elements of slow muscle fibres are activated is not known, but the evidence suggests that there is a close link between the activity and the state of polarization of the membrane. It was shown in the previous paper that a s.n. tetanus produced a 'depolarization plateau'. There is also a maintained contraction in these elements in the region of the cathode during the passage of direct current. Further, depolarization by drugs produces an 'active' phase of contraction which may persist for half an hour or longer; this can be immediately relaxed by an in-going (anodal) current which restores the membrane charge. The conclusion drawn is that in the slow fibres the coupling process between membrane changes and contractile elements remains unbroken for long periods, in contrast to the twitch fibres which have acquired the property of breaking the link more rapidly, so that contraction comes to an end even if the membrane depolarization persists.

Another difference in the coupling mechanism in twitch and slow muscle fibres is seen at the junctions. End-plate potentials, even of 20–25 mV, obtained with internal leads (curarized, or in the absence of drugs), may not cause any detectable local contraction in twitch fibres, and only at a critical size do they set up the propagated impulses and twitches. No such critical threshold between surface charge or extent of depolarization is seen in slow muscle fibres. With the gradual growth of small junctional potentials a progressive contractile activation occurs.

Function of slow fibres. The functional role of slow muscle fibres in the body clearly suggests itself from a variety of data. It was shown that these fibres tend to be reflexly activated even when the muscles are otherwise 'at rest'. In decapitated frogs appropriate afferent stimulation can produce considerable slow muscle fibre tension without involving the twitch system and phasic movement. Slow muscle fibres are active, however, also during twitch activity. They are more economical, in that stimulation at low frequency can 'hold' relatively large tensions originally produced by twitches. In this connexion

a functionally useful synergism between twitch and slow muscle fibres has been demonstrated (Fig. 6*a*).

It has been pointed out repeatedly that 'tonic' muscles (and therefore slow muscle fibres) may serve in particular the clasp reflex. In this context it should be noted that the present findings refer to males and females at all seasons, to flexors and extensors. Slow muscle fibres are plentiful in eye muscles which can hardly play a direct role in the clasp reaction. Their function may reasonably be given a wider interpretation as serving the requirements of general postural activity. In a different area of muscular studies mechanical changes associated with local potentials have been seen in smooth muscles and have been tentatively called 'resting tonus' by Bozler (1948). To account for such activity, however, no special muscular elements need to be postulated.

Histologically the studies of Krüger and co-workers have to be noted. They find in frogs two types of muscle fibres, those with 'Fibrillenstruktur' and 'Felderstruktur'. The latter type occurs in muscles, or parts of muscles, e.g. the slow portion of the iliofibularis, where small-nerve innervation (and slow muscle fibres) is now known to exist. 'Fibrillary' structure of muscle fibres predominates or is exclusively found in other muscles which receive large-nerve innervation, like the sartorius or the twitch portion of the frog's iliofibularis. It might seem reasonable to correlate these observations and suggest that the fibres with 'field' structure correspond to the slow muscle elements. A summary of Krüger's (1952) extensive studies has recently appeared. Krüger also finds a similar histological grouping of fibres in other animals, including cats. Although a careful search was made in several muscles, including the cat's soleus which has Krüger's 'field' structure, no muscle fibres of analogous *functional* characteristics to the slow fibres could be found. They all gave propagated twitch responses. The mammal does, however, possess a small-nerve system subserving reflex function (Kuffler, Hunt & Quilliam, 1951; Hunt, 1951); but all the small-nerve fibres (3–8 μ) in the lower extremities innervate the intrafusal fibres of the muscle spindles and modulate the rate of firing of their afferent impulses, which in turn take part in controlling posture by regulating twitch reflexes. The intrafusal muscle elements contained in mammalian spindles appear to be analogous to the frog's slow muscle fibres in several respects. Both muscle types are innervated by small diameter nerves and are activated by a graded facilitation mechanism and influence posture. Intrafusal muscle fibres in the cat presumably give local contractions during excitation (Kuffler & Hunt, 1952). The frog's slow muscle fibres act *directly* in producing tension during posture, while the mammalian intrafusal muscle fibres produce negligible external tension but are able to excite sensory nerve endings within the spindle. By their reflex action they produce a large *indirect* effect on posture (Hunt, 1951; Kuffler & Hunt, 1952). Besides the functional analogies there seem to exist also pharma-

cological analogies (Hunt, 1952). In general the physiological behaviour of slow muscle fibres seems to stand somewhere between smooth muscle and twitch-skeletal muscle.

Identification of slow muscle fibres by means of their ACh contracture alone may not necessarily give unequivocal information. Several authors (see Wachholder & Nothmann, 1932) have studied the variability of the ACh response with seasons and they found that 'tonic' muscles become more sensitive and give bigger responses during the winter, while 'non-tonic' muscles may to some extent be converted to 'tonic' behaviour. From such experiments it was concluded that the same muscle fibres were able to behave in two different ways and become more or less 'tonic' according to either season or metabolic state. These findings and the recording methods were not repeated since our frogs were usually kept, even in winter, near room temperature for at least some days. In any event these considerations do not affect the basis on which the present crucial identification of slow muscle fibres is made, namely the physiological response with small-nerve stimulation and intracellular recording. The innervation pattern is definitively laid down and not subject to seasonal variations.

SUMMARY

1. Extending earlier studies, the contractile behaviour of the frog's slow skeletal muscle fibres in response to nervous, electrical and chemical stimuli has been investigated. Slow muscle fibres innervated exclusively by small-nerve fibres differ strikingly in many respects from twitch muscle fibres which are supplied by larger motor axons. In slow fibres the contractile system is activated locally at numerous points around multiple small-nerve junctions which are distributed over the fibre surface.

2. Repeated stimulation of small nerves is necessary to cause a significant tension rise in slow muscle fibres. In the iliofibularis at 20–22° C a stimulation frequency of 40–50/sec develops a maximal slow fibre tension of several grams which can be maintained for many minutes. Higher frequencies speed up the rate of tension rise. Relaxation after slow muscle fibre contraction is at least 50 to 100 times slower than after twitch fibre action. All the manifestations of slow muscle fibre activity are greatly influenced by initial muscle tensions and the methods of recording. These differences are demonstrated and discussed.

3. Tension caused by separate stimulation of slow fibres sums with tension produced by twitch fibres. Whenever slow fibres were found they were interspersed between twitch fibres. Synergistic interaction between the two contractile systems occurs (Fig. 6*a*).

4. Slow muscle fibres show specific electrical properties. Twitch fibres relax completely after single or repeated excitation by current pulses which are able

to cause pronounced residual tension in slow muscle fibres. If constant currents are passed through twitch fibres, first repeated propagated responses are set up followed by local shortening around the cathode. Within several minutes, however, the twitch fibres relax in spite of continued current flow. In contrast, with equivalent currents, slow fibres maintain tensions for the duration of current flow in tests up to one hour.

5. During immersion into depolarizing solutions slow fibre tension declines slowly. Twitch fibres relax even in the continued presence of isotonic KCl or strong acetylcholine (ACh) solutions. ACh assays can only be done on slow fibres.

6. The 'active' and 'passive' components in slow muscle fibre tension records, or during shortening, are analysed by the application of intermittent stretches. 'Active' tension or shortening is thought to be controlled by the membrane potential. It is concluded that the contractile mechanism in slow fibres 'accommodates' slowly to the continued membrane change, while the converse occurs in twitch muscle fibres.

7. The slow fibres appear to be responsible for many of the well-known 'tonic' phenomena, seen in frog muscles (Sommerkamp, Wachholder).

8. It is concluded that slow fibres play an important role in the general postural activity of the frog. It is noted that no similar mechanism in mammals has been found. These differences are discussed.

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